THERAPEUTIC STUDIES ON RINGWORM-INFECTED GUINEA PIGS*

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It is well known that frequently there is a lack of correlation between *in vitro* observations and *in vivo* results in the evaluation of fungicidal compounds. Ultimately all antimycotic agents must be tested on infected animals to establish their practical worth. An epizootic of ringworm among guinea pigs caused by *Trichophyton mentagrophytes* presented an opportunity to carry out therapeutic studies on a large group of infected animals. (1) The results of tests with 4 fungicidal preparations on spontaneously infected animals are presented in this paper.

MATERIALS AND METHODS

Observations by Fuentes (2) and by the writers indicate that dermatophytes can be isolated frequently from animals that appear to be clinically normal. On this basis it was decided that the criterion for effective treatment should be based on the elimination of pathogenic fungi from the host rather than on the mere healing of lesions. In order to achieve this objective, it was considered necessary to treat the entire body of the animal with the test compounds.

The following preparations were screened to determine their utility as dips: "Cerbinol",‡ a preparation containing neutral salicylic and benzoic esters of propylene glycol; "Naprylate",§ an aqueous solution of 20% sodium caprylate; "Ortho Tack Wash",‖ a powder containing 45% technical captan (N-trichloromethylmercapto-4-cyelohexene-1,2-dicarboximide); "10–10", a solution of 10 % tannic acid, 10 % salicylic acid and 2 % benzoic acid in 70% ethyl alcohol. Of these 4 compounds only "Ortho Tack Wash" was found to be non-toxic when used as a dip. Accordingly, this preparation was tested for its effectiveness in eradicating *T. mentagrophytes* from spontaneously infected guinea pigs.

The guinea pigs utilized in this study were of the English short-hair type, of mixed color, and weighed from 200 to 370 grams. Their average age was 6 weeks.

The "Ortho Tack Wash" was used at a dilution of 1:200 as recommended by the manufacturers in the treatment of cats and dogs. Fresh solutions of the drug were prepared for each dip. For treatment the test solution was poured into a deep pan and the guinea pigs were immersed in the solution and scrubbed well with a stiff brush.

The tests were carried out in 2 parts. In the first study 15 control animals, confined to one

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Note: The use of trade names is for the purpose of identification and does not constitute indorsement by the U. S. Public Health Service.

‖ California Spray-Chemical Corporation, Richmond, California

cage, and 17 test animals, also held in a single cage, were used. The test animals were dipped twice weekly in the drug solution over a period of 4 weeks. This group of animals was placed in a clean cage after each dip.

In the second study, 13 of the animals previously used as controls were subjected to treatment as cultural studies had indicated that their infections had not cleared up spontaneously. Nine additional infected animals were added to this group. These 22 animals were caged individually. Each animal was dipped in the drug solution once a week for 4 weeks. After each dip the animal was placed in a clean cage that had been washed thoroughly with a solution of 30% cresol.

All animals were tested prior to the treatment studies and at regular intervals during and following the treatment period. Since most of the lesions occurred on the nose or back, these areas were regularly selected for culture whether or not lesions were present. Two specimens were obtained from each animal, one from the nose and one from the back (usually near the tail). Hair specimens for culture were obtained, using sterile forceps, and were kept in clean paper envelopes before culturing. These were cultured separately. They were inoculated into tubes of cycloheximide medium (3) and held at room temperature for a minimum of one month before any were discarded as negative.

RESULTS

Treatment Study 1

At the beginning of the treatment study only 13 of the 15 control animals (87%) were positive for *T. mentagrophytes* as were 13 of the 17 test animals

TABLE 1. *Treatment studies on guinea pigs naturally infected with* Trichophyton Mentagrophytes

Study	Pre-Treatment			Treatment	Post-Treatment								
	T	+	%		T	+	%	T	+	%	T	+	%
					1 day			1 month			2 months		
Study no. 1													
Treated	17	13	76	Two dips weekly for 4 weeks. Total of 8 dips. Ortho Tack Wash diluted 1:200	17	1	6	16	9	56	16	16	100
	Lesions 5 (29%)				Lesions 0 (0%)			Lesions 1 (6%)			Lesions 1 (6%)		
Control	15	13	8		14	7	50	13	12	92	13	13	100
	Lesions 7 (47%)				Lesions 8 (57%)			Lesions 2 (15%)			Lesions 10 (77%)		
					3rd day			1 month					
Study no. 2													
Treated	22	20	91	One dip weekly for 4 weeks. Total of 4 dips. Ortho Tack Wash diluted 1:200	22	1	5	19	2	11			
	Lesions 6 (27%)				Lesions 2 (9%)			Lesions 0 (0%)					

T—Total Cultured; +—*T. mentagrophytes*; %—Percent Positive

(76%) (Table 1). The test guinea pigs were treated by dipping them twice weekly for 4 weeks in a 1:200 solution of "Ortho Tack Wash."

On the first day after the 4-week treatment period, only one of the treated animals yielded a culture of *T. mentagrophytes*. However, the number of positive animals increased to 9 one month later and finally to 16 two months after treatment had been discontinued. Thus, following treatment a greater number of animals were found to be infected than at the start of treatment. At the end of the study all 15 of the control animals were positive for *T. mentagrophytes*.

It is important to note that during the treatment and post-treatment periods the animals were kept under crowded conditions in two cages. Thus, there was ample opportunity for *T. mentagrophytes* to pass from animals that were not entirely free of the parasite to those that may have been cleared.

Taking advantage of the fact that all of the control animals were positive at 28–30 weeks of age, they were kept under observation to determine whether spontaneous cure would occur. These animals were placed in groups of 2–3 in clean cages and cultured periodically over a 6-month period. No cultural evidence of spontaneous cure was obtained as all but one animal yielded cultures of *T. mentagrophytes* at the end of this time. During this period of observation it was noted that as most of the animals grew older their lesions tended to disappear. In many, however, new lesions appeared on different areas of their bodies. The absence of lesions, however, was found to be a poor criterion of absence of infections, as in all cases the fungus was isolated from these normal-appearing animals.

Treatment Study II

In this experiment 22 infected guinea pigs were used. Three days following treatment only one animal was positive; after two months only two guinea pigs gave positive cultures. All the other animals remained negative for several months. The healing that followed treatment was very striking. None of the animals had lesions one month following treatment.

The results indicated that treatment markedly reduced infection and healed the lesions in all the test animals. However, complete eradication of *T. mentagrophytes* was not accomplished.

DISCUSSION

Ringworm in guinea pigs is apparently more common than is realized. Animals free of clinical signs of infection harbor parasitic fungi and serve to spread infection in animal colonies.

Treatment of guinea-pig ringworm, thus, may prove to be fruitless if only those animals with obvious signs of infection are treated. Search for carriers should be carried out by routinely culturing the nose and tail areas of all apparently normal animals. All animals found to be infected should be treated and segregated until proved by culture to be free of dermatophytes.

The present study revealed the need to treat the whole animal rather than just the lesions in order to free the animal of dermatophytes. However, certain precautions must be taken. All drugs must be tested for toxicity prior to use over

large areas of the body. Of 4 drugs screened in this study, only one was non-toxic to guinea pigs immersed in solutions of the fungicide. Infected animals must be maintained in individual cages and placed in sterilized cages following each treatment. If this precaution is not taken, infection will spread from incompletely cured animals to those freed of the infecting agent. The only reliable criterion of cure is absence of dermatophytes as demonstrated by repeated negative cultures. Mere clearing of lesions is not a sound basis of determining effective treatment.

The preliminary experiments carried out to test the toxicity of the 4 test drugs revealed points of practical importance. During an epizootic which occurred in the animal colony, 500 infected guinea pigs were dipped in "Ortho Tack Wash" solution. This procedure was discontinued following the first dip because several of the animals lost weight and some died of pneumonia. Dipping or spraying may cause complications, especially during the winter months.

The second point of practical importance concerns the toxicity of the preparation used. Some of the preparations used for the treatment of ringworm are very toxic and cannot be used as dips or sprays. Apparently this is not generally known, in spite of the fact that they are recommended only for topical use by the companies that prepare them.

In one experiment, in which the 4 drugs were tested, 100 guinea pigs were dipped daily for 5 consecutive days, and 30 were used for controls. No deaths occurred among the 30 control animals; however, among the treated animals, 5 died after the first dip, 33 after the second dip, and 70 were dead by the end of the study. Naprylate caused death quite rapidly. All of the animals died shortly after the second dip in it. Cerbinol was slower in its action. One-third of the animals died after the fourth dip, and all but 2 were dead shortly after the fifth dip. Histopathological studies on tissues from 25 of the treated animals that died indicated that those animals that succumbed during the first two days of treatment did not develop lesions. These animals probably died of systemic anoxia and medullary paralysis. Animals that died after the second day of treatment showed pneumonia, hepatic lesions (fatty degeneration, focal necrosis, or abscesses) and glomerulonephritis. Tissues from 5 of the control animals showed no evidence of spontaneous disease.

SUMMARY

Therapeutic tests of a fungicidal compound were carried out on a group of guinea pigs spontaneously infected by *Trichophyton mentagrophytes*.

Eradication of the dermatophyte from the host's body rather than clearing of lesions was established as the criterion of cure. The basis for this was the knowledge that clinically normal guinea pigs harbor dermatophytes and, thus, serve to spread and maintain pathogenic fungi in animal colonies.

To accomplish this objective the entire body of the animal was treated by immersion into solutions of the test drugs. Of 4 drugs tested only one ("Ortho Tack Wash") proved to be non-toxic when used in the manner described above.

Twenty of 22 guinea pigs were freed of *T. mentagrophytes* after 4 dips, spaced at weekly intervals, in a 1:200 solution of "Ortho Tack Wash."

Infected animals had to be maintained in individual cages and placed in sterilized cages following each treatment. This precaution was necessary to prevent spread of infection from incompletely cured animals to those freed of the infecting agent or reinfection from contaminated cage debris.

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